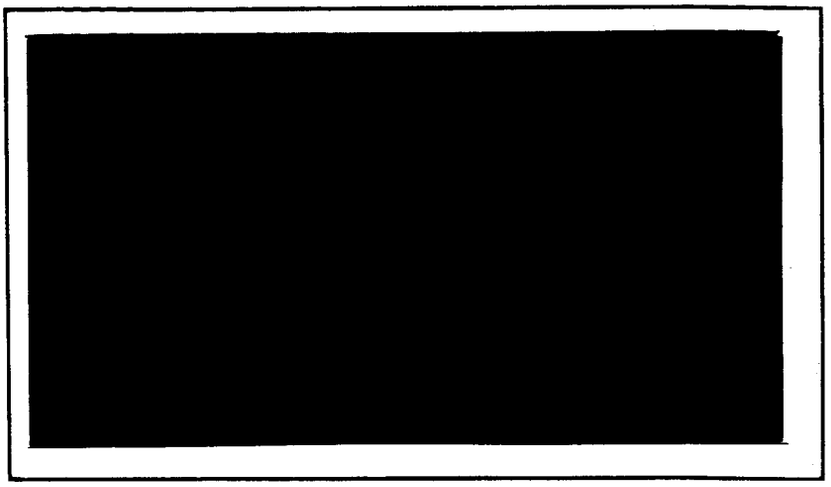

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Quarterly Progress Report

Covering Period: July 1 - September 30, 1964

Study of the Normal Fecal Bacterial Flora of Man

RAC 931-5

Prepared Under Contract NASw-738

by

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Quarterly Progress Report (July, August, September, 1964)
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INTRODUCTION

The work during this quarter was primarily concerned with isolation of bacteria from normal healthy males and the study of their carbohydrate metabolism. A rapid morphological type culture screening was also set up in addition to the regular type culture screening.

MORPHOLOGICAL TYPE CULTURES

Microscopic examination of fecal dilutions revealed a number of readily distinguishable types of bacteria which recurred frequently. These were designated by alphabetical letters to differentiate them from the physiological type cultures which were designated as types FA-1, FA-2, etc. These types are shown in Table I. Type A was a very short gram variable rod or coccus in short chains. Some cultures showed characteristic swelling in the chain. Type A was morphologically similar to FA-9 and FA-10. Type B was a short slender gram variable rod in pairs and often formed a "V" arrangement when the cells divided. This type was morphologically similar to types FA-1 and FA-14. Type C was a large lanceolate coccus in chains and was usually gram negative. Type D was a gram negative large fat rod in pairs sometimes with slightly pointed ends. Type E was an extremely pleomorphic gram positive rod and resembled fecal anaerobe types FA-4, FA-5 and FA-11. This culture was probably Actinomyces bifidus. Type G was a typical Fusobacterium often showing central swellings. There were indications that type C was a variant of type G. Type I was a typical streptococcus occurring in pairs. Type J was a gram negative short fat rod similar to Escherichia coli. Type K was a gram variable rod occurring typically in a parallel palisades arrangement. This type was similar to type U except that the latter usually occurred in pairs, longitudinally arranged. Type L was a long thread-like gram variable rod. Type M was a gram variable long thick rod usually showing an angular distortion to the cell. Type N was a gram positive rod usually quite

TABLE I

MORPHOLOGICAL TYPES OF FECAL ORGANISMS

A. Gram ± short rods in pairs short chains sometimes with coccoid swellings

B. Gram ± short to medium slender rods singly and in pairs

C. Gram ± large lanceolate cocci chains

D. Gram ± large fat rods or cocci in pairs

E. Gram ± medium pleo rods in pairs many forms, always shows pleomorphism

G. Gram ± pointed rods, longer than C, sometimes shows central swelling

I. Gram ± elongated cocci pairs and chains strep

J. Gram - short fat rods - coli

K. Gram ± medium rods singly, pairs and rafts no pleomorphism

L. Gram ± long threadlike often irregular staining

M. Gram ± long rods, thicker than L sometimes shows banded staining

N. Gram + medium reg. rods (like C. Welchii)

O. Gram ± rods larger than A.

P. Gram + micrococcus

Q. Gram - med rod pairs slightly curved

R. Gram ± very large cocci P.

S. Gram - slender curve rod

T. Gram + very large fat rod

U. Gram ± med rods in pairs may be same as K.

V. Gram - short fat rods in chains

regular in morphology. Type O was a very short gram variable rod occurring in pairs being differentiated from type B mainly because of size. Type P was a typical micrococcus. Type Q resembled a Selenomonas. This was a gram negative gently curving rod in pairs; the rods forming an arc. Type R was a large round gram variable coccus in pairs. This organism was about five times the diameter of type I and was usually gram negative. Type S was a very small gram negative vibrio. The cells on division formed the figure "S". Type T was a very large gram positive rod. Type V was a gram negative short fat rod in chains.

The use of these morphological type cultures greatly facilitated the description of microorganisms under the microscope. These types were not presumed to represent pure cultures and as pointed out above some may be variants of others.

SUBJECT CULTURING

The second round of culturing was continued on available subjects. Emphasis was placed on assessing reproducibility of the techniques used. In general it was found that the morphological types in duplicate dilution tubes were very dissimilar. These results are shown in Table II. These results indicate that certain types of bacteria occur in nearly the same numbers and that the ten-fold dilutions used are insufficient to separate them.

Several subjects were cultured using standard techniques. Duplicate series were run using screw cap tubes in place of the tubes with loose-fitting caps. It was hoped that the screw cap tubes would provide a longer duration of anaerobiosis since results indicated that the regular broth lost most of its reducing power after two days. Methylene blue reduction tests indicated that the screw cap tubes did indeed retain anaerobiosis for periods up to ten days. The cultural results (Table II) indicated that the types of bacteria were similar in the two series.

Other comparative studies were made using fecal samples that had been kept at room temperature for five hours. These were compared with the original fresh fecal culturing. The results did not indicate any significant change in morphological types recovered.

FECAL ANAEROBES TYPE CULTURES

The FA type cultures were studied further in an attempt to identify them according to Bergey's manual. Preliminary examination of these cultures indicate that FA-2 and FA-7 may belong to the genus Sphaerophorus. FA-3 and FA-15 are in the genus Fusobacter; FA-13 is in the genus Veillonella; FA-9, FA-10 and FA-12 are in the genus Dialister and types FA-4, FA-5, FA-6 and FA-11 are most likely Actinomyces bifidus. The other types are presumably Bacteroides.

TABLE II

REPRODUCIBILITY OF OCCURRENCE OF MORPHOLOGICAL TYPES

Sample	Dilution Tube	Replicate #			
		1	2	3	4
A-Open tubes	5	UBDECQG	UADGEB	GUQBL	ABGEDQU
	6	V	U	ABLGU	GLD
	7	E	GL	-	VG
A-Screw cap tubes	5	LEGDCV	DBECGUVQ	UDBALE	ULGBED
	6	IBEUJ	UDE	UBVJ	GBVD
	7	U	DL	L	V
B-Open tubes	5	GLBV	GLUV	GLV	GLV
	6	BVG	LG	GLJV	LB
	7	-	LG	G	GV
B-Screw cap tubes	5	LGV	BGVI	GLV	GOI
	6	LVG	VBG	BVG	OGV
	7	L	GL	GL	OG
C-Open tubes	5	L	GVAJQE	GULA	UGBARE
	6	GCABE	GB	UEIG	LUGE
	7	IG	_	V	G
C-Screw cap tubes	5	UGBQLV	UGQEA	EOGQ	EUCVG
	6	L	EGL	BEG	LG
	7	U	G	EB	L

MANOMETRIC STUDIES OF TYPE CULTURES

This quarter's work on the physiology of bacteria included a continuation of the April-June manometric fermentation balance studies. The purpose of these preliminary studies was to:

1. group type cultures on the basis of similar and different fermentation patterns,

2. ultimately assist in the taxonomy of the type cultures, especially the Lactobacilli,

3. quantatively confirm qualitative laboratory observations on fermentation, and

4. establish some of the major metabolic end products which accumulate as a result of carbohydrate fermentation.

Many end products from bacterial glucose fermentation have been reported in the literature. These include carbon dioxide and hydrogen gas, formic, acetic, propionic, butyric, lactic and succinic acids; glycerol, acetoin, butylene glycol, ethyl, isopropyl, and butyl alcohols; acetone, and many other compounds. Since a complete fermentation balance is both complex and tedious, it was decided to limit the preliminary screening of type cultures to manometric estimation of CO_2, H_2, lactic acid, and total carboxylic acid groups formed. However, Republic's gas chromatography facilities may be used for more difinitive studies later in the program.

Our approach to a preliminary fermentation balance then, consisted of a continuation of the manometric studies outlined in the last quarter. Most of the type cultures were tested for their ability to ferment glucose, lactose, maltose and starch. These carbohydrates were chosen because they are common in the diet and are also particularly useful for differentiating the fecal anaerobes, especially Lactobacillus species.

A summary of the preliminary results from the manometric studies are given in Table III. It is obvious that FA-4, FA-5, FA-6, and FA-11 have similar fermentation patterns. This observation was not surprising, since they are considered to be similar on the basis of other criteria. Another significant observation is that starch was utilized at a higher rate than glucose. This is characteristic of Actinomyces bifidus. Further work will complete the table and clarify the relationships of the other type cultures.

TABLE III

SUMMARY OF MANOMETRIC DETERMINATION
OF CARBOHYDRATE FERMENTATION

FA	Lactose	Maltose	Glucose	Starch
1	0	0	0	0
2	A	A	A	
3	A, CO_2	A, CO_2	A, CO_2	A
4	A	A	A	A
5	A	A	A	A
6	A	A	A	A
7	0	0	0	0
9	A	A	A	
10	A, CO_2	0	CO_2	0
11	A	A	A	A
12	A	0	A	0
13			0	
14			CO_2, H_2	
15			A	
16			A	

A = Organic acids formed

PROJECT PERSONNEL

Personnel who have been working on the program are Dr. Lorraine S. Gall, Charles Huhtanen, Norman Richards, Fay Ames and Shirley Dunwoody.

HOURS EXPENDED: (July 1 - September 30, 1964)

Professional: 454
Technician: 98

PROJECTED WORK

Future work will include determination of some remaining aspects of the fermentation balances, including estimation of lactic acid formed from the selected carbohydrates. Several other organic acids may also be determined by means of our gas chromatograph.

Lorraine S. Gall, Ph. D.

LSG/bs